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Reconstructing the geographic origin of the New World jays

Sumudu W. Fernando^{a*} , A. Townsend Peterson^a  and Shou-Hsien Li^b

^aBiodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS, USA;

^bDepartment of Life Science, National Taiwan Normal University, Taipei, Taiwan

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We conducted a biogeographic analysis based on a dense phylogenetic hypothesis for the early branches of corvids, to assess geographic origin of the New World jay (NWJ) clade. We produced a multilocus phylogeny from sequences of three nuclear introns and three mitochondrial genes and included at least one species from each NWJ genus and 29 species representing the rest of the five corvid subfamilies in the analysis. We used the S-DIVA, S-DEC, and BBM analyses implemented in RASP to create biogeographic reconstructions, and BEAST to estimate timing of NWJ diversification. Biogeographic reconstructions indicated that NWJs originated from an ancestor in the Eastern Palearctic or Eastern + Western Palearctic, diversified in Mesoamerica and spread subsequently to North and South America; the group has been diversifying in the New World since the late Miocene.

Keywords: New World jays; Corvidae; biogeographic origin; divergence times

Introduction

The New World jays (NWJs) are a monophyletic group of corvids [1,2], presently considered to comprise seven genera (*Aphelocoma*, *Calocitta*, *Cyanocitta*, *Cyanocorax*, *Cyanolyca*, *Gymnorhinus* and *Psilorhinus*); the ~36 species [3,4] represent the product of a radiation across much of the Americas [1]. The group has long been a focus of research in behavioral ecology, in light of complex behavioral repertoires, particularly as regards social behavior [5–7].

The origins of corvids can be traced to Australia, from where the ancestor of the family dispersed to Asia, followed by radiations in Asia, Europe and elsewhere [8]. Ericson and colleagues [9–11] provided evidence that the origin of the oscine passerines, to which the Corvidae belongs, dates to the split of the Australo-Papuan tectonic plate from Antarctica in the early Tertiary, ~53M years ago. Thus, the replacement of the early Tertiary rainforests in Australia by drier vegetation may have placed presumably forest-adapted early corvids in more open habitats, resulting in a new radiation that led to the present global distribution of the family. However, a recent study [12] estimated the age of the family at ~20M years. Colonization of the New World by corvids occurred more recently, apparently via a trans-Beringian route [8]. The ancestor of NWJs, thought to be jay-like lineages related to *Cissa* and *Urocissa*, reached North America 10–8M years ago, in the Miocene [13]. Once in the Americas, a rapid radiation in South America by the early Pliocene, was apparently followed by a secondary radiation in North America [1]. This hypothesis was not supported by the later analyses

of Bonaccorso and Peterson [14], who offered support for a hypothesis that the NWJ ancestor was in Mesoamerica or North America + Mesoamerica and radiated into North and South America independently. The origins of this diverse clade are of considerable interest in light of questions about the biogeography of Asian and American tropical groups [15], morphological innovation [2] and the evolutionary origins of social behavior [2].

Several previous studies have examined the evolutionary history, phylogeny and biogeography of the NWJs, but invariably based on sparse representation of NWJ genera and limited outgroup sampling. De los Monteros and Cracraft [1] proposed a first hypothesis of intergeneric relationships in the group based on cytochrome b sequences. Ericson et al. [11] added markers but included only single species per genus. Saunders and Edwards [16] examined mitochondrial DNA control region (CR) variation among representatives of all NWJ genera. Finally, Bonaccorso and Peterson [14] derived a multilocus phylogeny with much-improved NWJ species representation and included a historical biogeographic analysis that concluded that NWJ origins were in Mesoamerica or Mesoamerica + North America.

These previous studies, however, did not provide sufficient detail in terms of representation of taxa of NWJs and other corvids to permit thorough understanding the geographic origin of NWJs or to identify their closest corvid relatives. The key deficiency has been in terms of representation of taxa from the deepest branches of the corvid phylogeny; thus, we here derive a denser phylogenetic hypothesis by deriving DNA sequences for

*Corresponding author. Email: sumudu@ku.edu

these early lineages, placing several species of treepies, blue magpies and green magpies in a molecular phylogeny for the first time, to permit more robust biogeographic analyses. In light of this new data set, we discuss geographic origins of NWJs and offer a new hypothesis.

Methods

Taxon and gene sampling

Taxon sampling included at least one species from each NWJ genus, 29 species representing the rest of the five corvid subfamilies and *Lanius* as a definitive outgroup (see Appendix 1 for a summary). Frozen tissue samples (muscle tissue) were obtained from the American Museum of Natural History, Louisiana State University Museum of Natural Science, National Taiwan Normal University and the University of Kansas Natural History Museum. We also added DNA sequences from GenBank from previous studies [1,11,14,17–34] that overlapped our sequenced gene regions.

DNA sequences were obtained for three mitochondrial genes (cytochrome b, or Cytb; nicotinamide adenine dinucleotide dehydrogenase subunit 2, or ND2; and nicotinamide adenine dinucleotide dehydrogenase subunit 3, or ND3), and three nuclear introns (the seventh intron of the beta-fibrinogen gene, or β fib7; the second intron of the myoglobin gene, or Myo2; and the fifth intron of the transforming growth factor β 2, or TGF β 2). Genes were amplified using the following primers: Cytb, L14851 [35] and L428 and H494 [36]; ND2, L5215 and H6313 [37]; ND3, L10755 and H11151 [38]; β fib7, FIB-B17U and FIB-B17L [39]; Myo2, Myo2 [40] and Myo3f [41]; and TGF β 2, TGF5 and TGF6 [35].

DNA extraction, PCR amplification, sequencing, alignment and model selection

DNA was extracted from frozen tissues using proteinase K digestion under manufacturer's protocols (Qiagen DNeasy tissue kit). PCR amplifications were performed using Promega GoTaq Flexi DNA polymerase. We employed a touchdown protocol for the amplification of ND2 and ND3, with annealing temperatures of 55 and 50°C, respectively. Constant annealing temperatures of 58, 50, 59 and 56°C were used for Cytb, β fib7, Myo2 and TGF β 2, respectively. PCR products were sent to Beckman Coulter Genomics (Danvers, MA) for purification and final sequencing.

Sequences were cleaned, assembled and aligned in Geneious 8.1.6 (Biomatters, Auckland, New Zealand) and checked against an automated alignment in MUSCLE [42]. Sequence data were partitioned by codon position for mitochondrial DNA and by gene for nuclear introns. We used MrModeltest 2.3 [43] to identify appropriate substitutional models for each partition under the Akaike information criterion (AIC).

Phylogenetic analysis

Single-gene matrices were concatenated using Geneious 8.1.6 to produce an overall mitochondrial data set, a nuclear intron data set and a total combined matrix. Phylogenies were estimated with Bayesian analysis (BA) and maximum-likelihood (ML) inference methods. GARLI v2.01 [44] was used for the maximum-likelihood analysis. We performed 100 independent analyses and chose the tree with the best likelihood score. Nodal support values for topologies were evaluated via 500 nonparametric bootstrap replicates [45]. The 50% majority-rule consensus tree was acquired in PAUP* 4.0 [46]; 70% bootstrap support was considered as indicative of solid nodal support [47,48].

Bayesian phylogenetic estimates were obtained in MrBayes 3.2.2 [49–51]. Two independent Markov chain Monte Carlo (MCMC) runs of 2×10^7 generations using four chains (temp = 0.1) per run were conducted. We sampled trees every 2000 generations and discarded the first 25% of the trees as burn-in. We used TRACER 1.6 [52] to assess convergence of parameter estimates and posterior probabilities. The remaining trees were summarized to obtain a 50% majority-rule consensus tree.

Ancestral area reconstructions

We employed S-DIVA (Statistical Dispersal-Vicariance Analysis), 'Bayes-Lagrange' or Statistical Dispersal-Extinction-Cladogenesis (S-DEC [53,54]), and Bayesian Binary Method (BBM) analysis implemented in RASP 3.2 [55,56] to reconstruct ancestral areas of corvid lineages. DIVA is an event-based method in which vicariance is assumed and includes potential contributions for dispersal and extinction by assigning a cost [57]. S-DIVA is an expansion of Bayes-DIVA implemented by Nylander et al. [58] that incorporates the entire posterior distribution of tree topologies, thus accounting for both phylogenetic and ancestral state uncertainty [55,56]. S-DEC model has been implemented using the source code for the C++ version of Lagrange [59]. This model gives the likelihood of all possible biogeographic scenarios estimated at a given node and summarizes biogeographic reconstructions across all user-supplied trees [56]. BBM is a statistical procedure in which a full hierarchical Bayesian approach is used to reconstruct ancestral distributions at ancestral nodes [60]; it is implemented in RASP [56], wherein the source code of MrBayes 3.1.2 is used to create the MCMC analysis.

We defined 8 geographic areas (A = North America, B = Mesoamerica, C = South America, D = Western Palearctic, E = Eastern Palearctic, F = Afrotropics, G = Indo-Malaya, H = Australia). We assigned each species included in the phylogenetic analysis to a region or regions, based on published range maps [61]. S-DIVA, S-DEC and BBM analyses were conducted using 10,000 postburn-in trees derived from the overall combined data matrix from the Bayesian Markov chain Monte Carlo

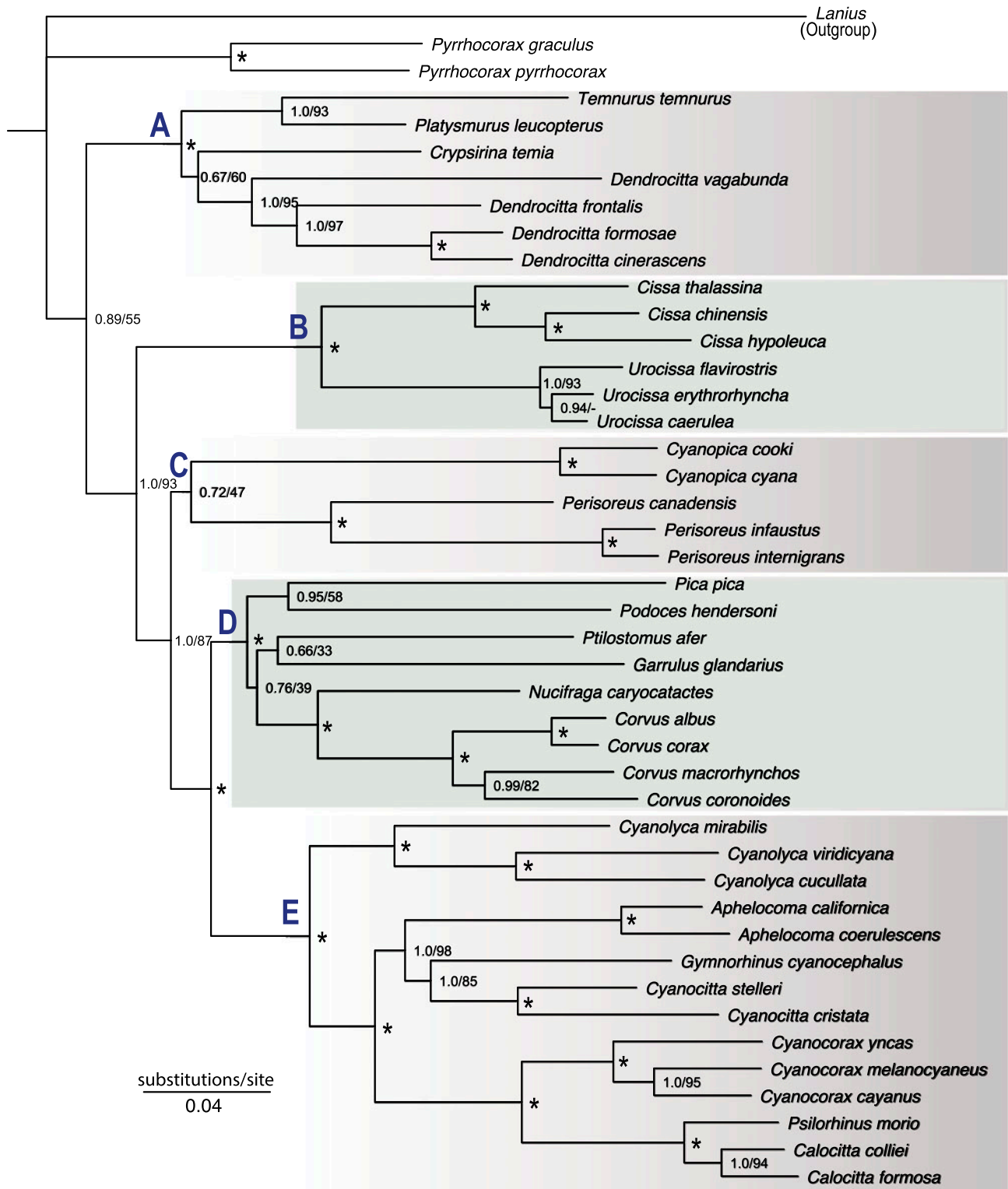


Figure 1. Phylogeny of Corvidae based on Bayesian and Maximum Likelihood analyses of the combined data-set. Numbers at the nodes refer to Bayesian posterior probability/ML bootstrap support. Asterisks indicate 1.0 posterior probability and 100% bootstrap support. Letters at nodes indicate five major clades of the family.

(MCMC) procedure implemented in BEAST 1.8.2 [62]. For the S-DEC analysis, we considered the probability of dispersal between areas as equal, and all values in the dispersal constraint matrix were set to 1. BBM analysis used the maximum clade probability tree (condensed

tree) from the BEAST analysis and included two independent runs of 10 chains (temp = 0.1) that ran for 1M generations, sampling every 100 generations, but discarding the initial 25% as burn-in. We used the F81 + G evolutionary model for the analysis, based on the results of

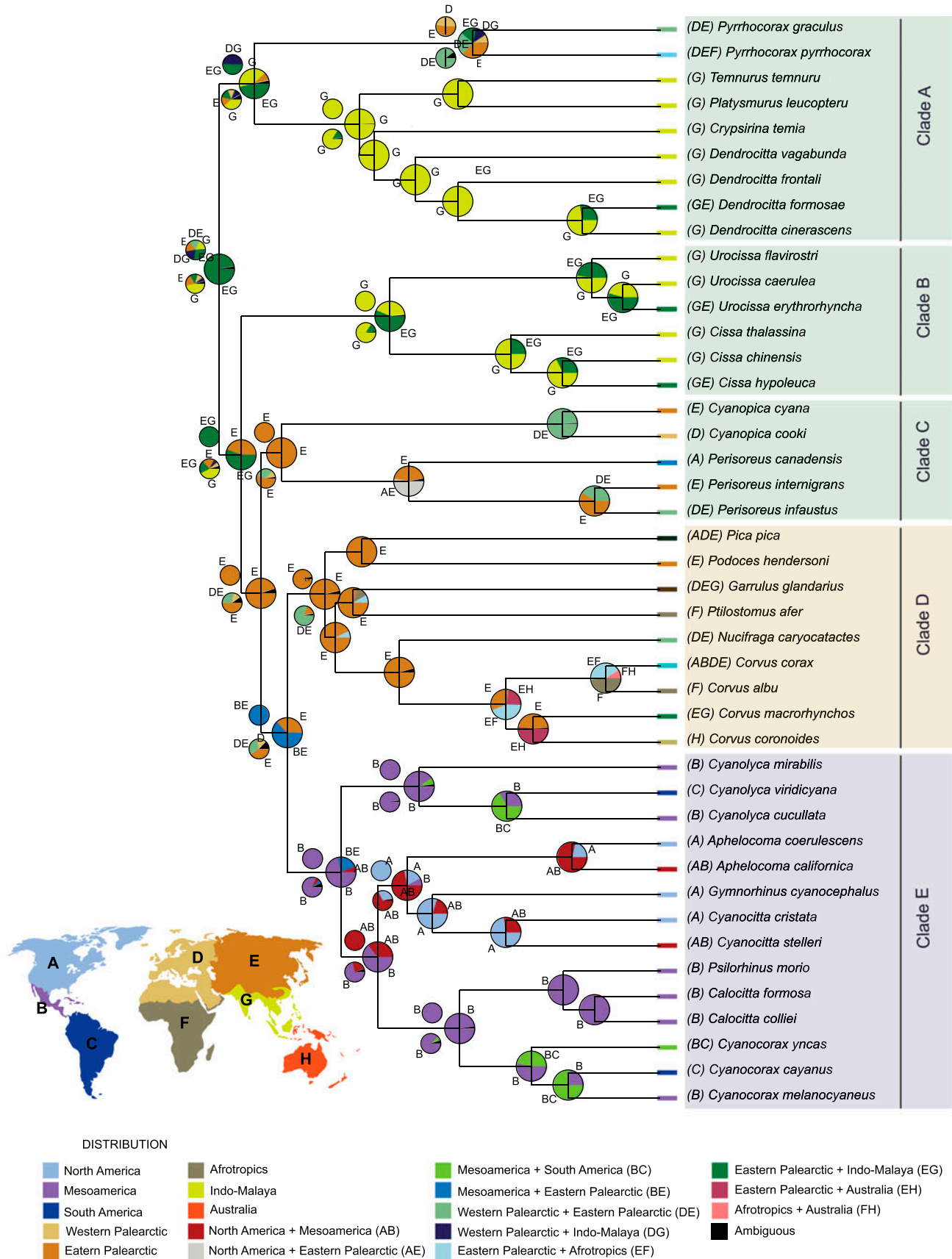


Figure 2. Reconstruction of the ancestral area of lineages of Corvidae. Pie charts indicate probability of various ancestral area combinations. Pie charts at nodes were derived from S-DEC analysis; the smaller charts above and below at the nodes obtained from S-DIVA and BBM analyses, respectively. Ancestral areas are shown only for basal nodes. The New World jay clade (clade E), its sister clade (clade D) and all other basal clades (C, B and A) of corvids, are outlined in light blue, orange and green boxes, respectively.

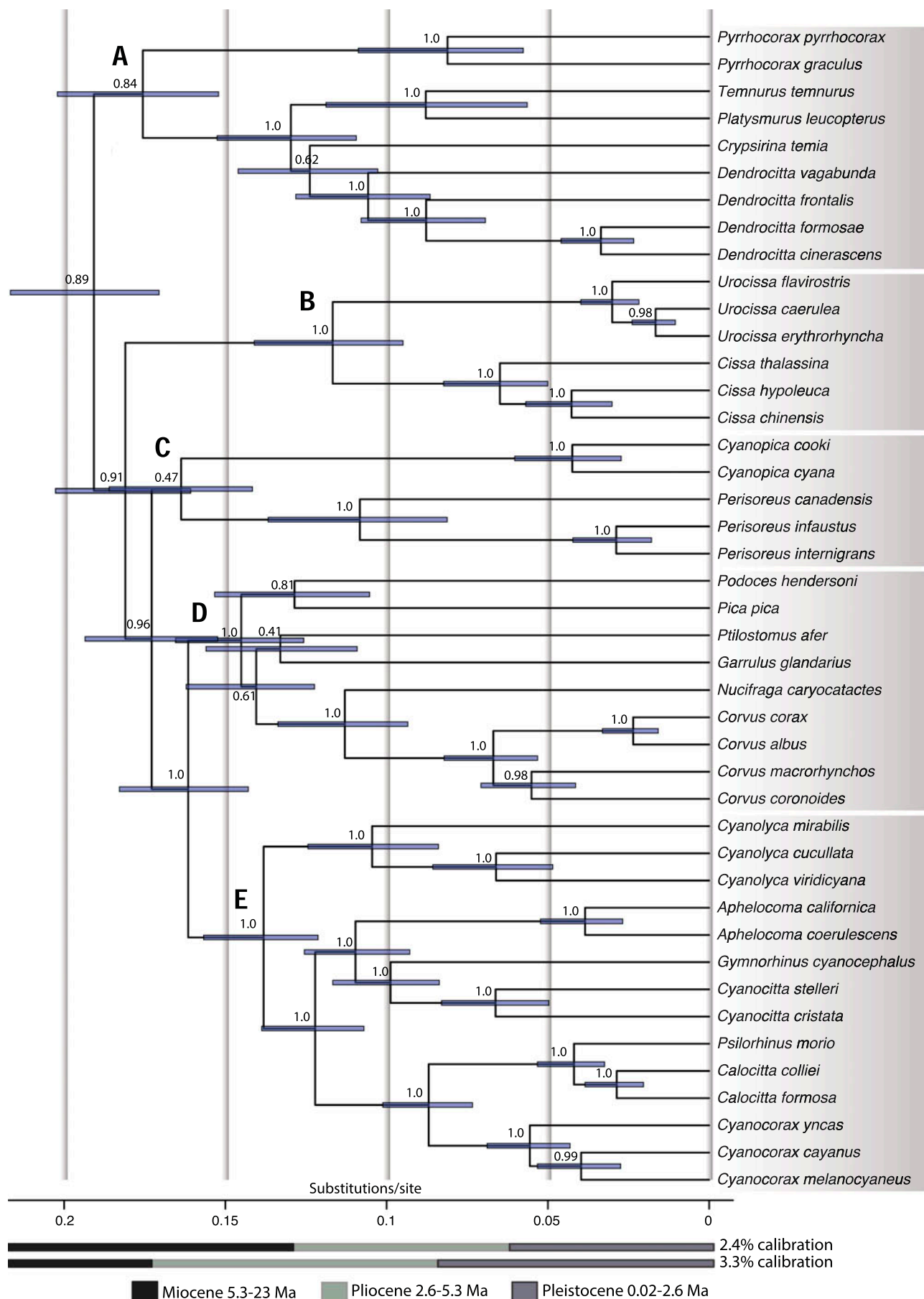


Figure 3. Approximate timeframe for the diversification of the Corvidae, with particular focus on New World jays. Time scales are based on ND2 substitution rates of 2.4% and 3.3% per lineage per million years. Bars at nodes indicate the highest posterior density (HPD) interval. Numbers at nodes refer to Bayesian posterior probabilities. Letters at nodes indicate the five major clades of the family.

the model testing described above. In all analyses, the maximum number of areas included in ancestral distributions was set to 2. We excluded the broadly distributed outgroup *Lanius* from these analyses.

Timing of diversification

The fossil record for passerine birds is scarce and many described fossils lack information necessary for use as informative calibration points [63]. Thus, we estimated the time of diversification of NWJs using previously published ND2 substitution rate priors using BEAST 1.8.2 under a relaxed clock model. We unlinked nucleotide substitution models, and used MrModelTest 2.3 [43] to obtain best-fitting substitutional models. We linked clock and tree models, used a birth-death speciation process and ran them for 5×10^8 generations, sampling every 50,000th generation. Convergence was examined in TRACER [52]; all effective sample size (ESS) values were > 200 . We discarded the initial 25% of samples as burn-in. Calibrating a phylogeny based on empirically determined mitochondrial DNA substitution rates offers only a rough estimate of actual time, though lacking fossils within the clade, such an external rate calibration offers the only option for linking to actual dates [64]. ND2 is widely studied in ornithology [65]; we used two empirically estimated substitution rate priors derived from ND2 substitution rates (2.4 and 3.3%/lineage/million years) for Hawaiian honeycreepers [66] that were derived based on three recently obtained geologic calibration points, to estimate divergence times for various corvid lineages.

Results

Topologies inferred via ML and BA analysis were highly congruent for the concatenated mitochondrial and nuclear sequence datasets (Figure 1; also see Appendix 2 for concatenated mitochondrial, and nuclear datasets). Results from the BEAST analysis were similar to the ML and BA topologies, except that *Pyrrhonorax* was placed as sister to clade A in the BEAST analysis (posterior probability 0.84); ML and BA could not resolve the placement of this genus. In general, these mostly congruent phylogenetic hypotheses all agreed in identifying five clades within the corvids: A, including *Temnurus*, *Platysmurus*, *Crypsirina*, and *Dendrocitta*; B, including *Cissa* and *Urocissa*; C, including *Cyanopica* and *Perisoreus*; D, including *Pica*, *Podoces*, *Ptilostomus*, *Garrulus*, *Nucifraga* and *Corvus*; and E, including *Cyanolyca*, *Aphelocoma*, *Gymnorhinus*, *Cyanocitta*, *Cyanocorax*, *Psilorhinus* and *Calocitta*. The NWJ clade (clade E) was placed as sister to clade D with strong support (posterior probability 1.0, 100% ML bootstraps). Monophyly of the clades C, D and E was highly supported (1.0, 87%), although the sister relationship of *Perisoreus* and *Cyanopica* was ambiguous (0.72, 47%). Placement of *Podoces* as sister to *Ptilostomus* [11] was not well-supported; in contrast, a sister relationship between *Podoces* and *Pica*

was better-supported, with a posterior probability of 0.95% and 58% bootstrap support.

S-DIVA, S-DEC and BBM ancestral area reconstructions for NWJs produced congruent results, estimating Mesoamerica as the most probable ancestral area for the common ancestor of clade E (Figure 2). According to S-DIVA analysis, Mesoamerica + Eastern Palearctic was the most probable ancestral area ($p = 1.0$) for the common ancestor of clades D and E. S-DEC also showed Mesoamerica + Eastern Palearctic as the most probable ancestral area ($p = 0.63$), and Eastern Palearctic was the other possible area ($p = 0.37$). BBM results were equivocal, with four possible ancestral areas: Eastern Palearctic ($p = 0.40$), Eastern Palearctic + Western Palearctic ($p = 0.31$), Western Palearctic ($p = 0.16$) and ambiguous ($p = 0.13$). Because all NWJ lineages were connected to American areas, uncertainty in phylogenetic arrangements within clade C had no effect on our reconstructions of areas of lineages ancestral to the NWJs.

BEAST relaxed-clock analysis based on the two substitution rate priors indicated that the time to the most recent common ancestor for NWJs was 5.8–4.3M years. The split of the NWJ clade (clade E) from its sister clade (clade D) was estimated at 6.7–5.0M years (Figure 3). Our analysis estimated that the Corvidae likely originated in the late Miocene, 8.0–5.9M years ago.

Discussion

Our biogeographic analysis was based on denser taxonomic sampling of the early branches of the corvid phylogeny than had been available in any previous study. Our results provided solid evidence regarding the geographic origin of NWJs. Mesoamerica + Eastern Palearctic or Eastern Palearctic was consistently identified as the most probable ancestral area for the ancestor of the NWJ and its sister clade; thus, the lineage probably reached the New World from the Eastern Palearctic through a trans-Beringian route. An interesting observation that resulted from the ancestral area reconstruction was the absence of the common ancestor of clades D and E in North America (Figure 2). A plausible explanation to this observation can be drawn from studies on other taxa: for example, Fox [67], based on mammalian fossils, documented aridification of North America during the late Miocene, thus linking it with increased seasonality. Similarly, Kohn and Fremd [68] documented decreased faunal diversity in western North America in the late Miocene, which they postulated to coincide with increased aridity and seasonality. The ancestor of clades D and E probably largely bypassed North America during the late Miocene, perhaps using western coastal habitats under the climate-stabilizing effects of the Pacific Ocean that may have offered temporary corridors for passage from the Eastern Palearctic to Mesoamerica. Hence, early cladogenesis—at least among extant taxa—of the NWJ lineage probably took place either in Mesoamerica or in the Eastern Palearctic.

Our results indicated that the NWJs are most closely related to a clade comprising *Pica*, *Podoces*, *Garrulus* and others. Ericson et al. [11] identified Indo-Malaya as the ancestral area of origin of these taxa. In another study, De los Monteros and Cracraft [1] recovered *Perisoreus* as the sister taxon to the NWJ clade. Additionally, Craig et al. [13] described a close relationship between the NWJ ancestor and *Cissa* + *Urocissa*. Our results refute these hypotheses, with better taxonomic representation, a more robust multilocus data-set, and better support (Figure 1).

All of our analyses identified Mesoamerica as the most probable area for the most recent common ancestor of NWJs. This result narrows down the results of Bonaccorso and Peterson [14], which showed the ancestral distribution of the NWJs as either Mesoamerica or Mesoamerica + North America. The proposed origin of *Calocitta* + *Cyanocorax* + *Psilorhinus* in Mesoamerica, dispersing thence into South America [14], was supported in our analysis. However, our results were unclear as to whether the *Aphelocoma* + *Cyanocitta* + *Gymnorhinus* clade originated in North America vs. North America + Mesoamerica.

De los Monteros and Cracraft [1] proposed the idea that the origin of the NWJs was the result of a single dispersal event into the Americas, which could have taken place around the early Miocene. However, our time calibration estimates indicated a later (Miocene–early Pliocene) origin for the NWJs (Figure 3), in which the ancestor probably dispersed to the New World from the Eastern Palearctic, or Eastern + Western Palearctic, around 6.7–5.0 M years ago (Figure 3). This event could have taken place when a continuous coniferous belt linked Northern Asia and America across Beringia from mid-to-late Miocene to the late Pliocene [69], yet the timing of our reconstructions does not appear to coincide with any specific geological events that might have triggered the dispersal event.

Our time calibration estimated a late Miocene–early Pliocene origin for the most recent common ancestor (MRCA) of NWJs. This timing makes intuitive sense considering the absence of the MRCA in North America given the continent's increased seasonality and aridity by that time period, as stated above [67]. The time calibration further estimated the age of the family Corvidae at ~8.0–5.9 M years, substantially younger than the recent estimate of ~20M years in the phylogeny of corvid passerine birds [12]. However, we note that the timing of Corvidae–Laniidae split of the recent phylogeny of songbirds based on ~5000 loci by Moyle et al. [70] coincides with our estimate for the same node (not shown here). Moreover, our time estimates for the MRCA of the South American genera (2.8–2.0M years for *Cyanolyca*, and 2.3–1.7M years for *Cyanocorax*) make intuitive sense, since dispersal of these clades from Mesoamerica into South America should postdate the emergence of the Isthmus of Panama, around 2.8 M years ago [71]. However, we emphasize that our time

estimates are rough estimates of actual time and must be considered with caution.

Our results provide novel insights into the biogeographic origin of NWJs, offering a solid hypothesis based on different ancestral area reconstruction methods, backed by strong support from a robust, multilocus phylogeny. However, clearly, more complete taxon representation with genome-level sampling could perhaps improve support for some remaining ambiguous nodes in the phylogenetic hypothesis. Such an improved phylogeny would help to resolve ambiguities associated with some of our phylogenetic and geographic hypotheses, and could allow insight into other important questions, such as tropical-temperate transitions in the family.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

ORCID

Sumudu W. Fernando  <http://orcid.org/0000-0002-7477-8080>

A. Townsend Peterson  <http://orcid.org/0000-0003-0243-2379>

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Appendix 1. List of samples used in the study.

Genus/species	Institution/ Sample #	Locality	GenBank Accession					
			ND2	ND3	Cytb	Myo2	Fib7	TGF
<i>Apelocoma californica</i>	GenBank		AY030142	AY030162	AY030116	—	DQ912638	—
<i>Apelocoma coerulescens</i>	GenBank		DQ912601	—	U77335	AY395580	AY395598	—
<i>Calocitta colliei</i>	GenBank		DQ912603	—	DQ912591	—	DQ912640	—
<i>Calocitta formosa</i>	GenBank		DQ912602	—	U77336	AY395581	DQ912639	—
<i>Cissa chinensis</i>	KUNHM 30894	Vietnam: Kon Tum, Ngoc Linh Nature Reserve	KY492676	KY492613	JQ864486 ^G	KY492636	KY495261	—
<i>Cissa hypoleuca</i>	KUNHM 23241	Vietnam: Dong Nai; Vinh Cuu Reserve	KY492677	KY492614	—	KY492637	KY495262	KY492656
<i>Cissa thalassina</i>	LSUMZ 19185	Zoo/Captive	KY492678	KY492615	KY492609	KY492638	KY495263	KY492657
<i>Corvus albus</i>	GenBank		JQ024060	JQ023990	AY527262	—	—	—
<i>Corvus corax</i>	GenBank		JQ864446	—	JQ864490	KP070388	—	KP070515
<i>Corvus coronoides</i>	GenBank		JQ023957	JQ024031	AF197837	—	—	—
<i>Corvus macrorhynchos</i>	KUNHM 10955	Philippines: Cagayan; Calayan Island	KY492679	KY492616	HE805915 ^G	KY492639	KY495264	KY492658
<i>Crypsirina temia</i>	KUNHM 23219		KY495243	KY492617	KY492610	—	—	—
<i>Cyanocitta stelleri</i>	GenBank		KM115187	AY030159	KF208991	—	DQ912642	—
<i>Cyanocitta cristata</i>	KUNHM 2271	USA: Kansas; Shawnee	KY495244	KY492618	X74258 ^G	KY492640	KY495265	KY492659
<i>Cyanocorax cayana</i>	GenBank		GU144814	—	GU144857	—	DQ912650	—
<i>Cyanocorax yncas</i>	GenBank		KJ455397	—	KJ456245	KJ454784	GU144910	—
<i>Cyanocorax melanocyanus</i>	KUNHM 9260	El Salvador: La Laguna	KY495245	KY492619	GU144897 ^G	KY492641	KY495266	KY492660
<i>Cyanolyca cucullata</i>	GenBank		FJ598175	—	DQ912592	—	FJ598267	—
<i>Cyanolyca mirabilis</i>	GenBank		DQ912606	—	U77333 ^G	KY492642	DQ912644	—
<i>Cyanolyca viridicyana</i>	KUNHM 21176	Peru: Puno	KY495246	KY492620	AY701180	—	KY495267	KY492661
<i>Cyanopica cooki</i>	GenBank		—	—	—	—	—	—
<i>Cyanopica cyana</i>	LSUMZ 17007	Japan	KY495247	KY492621	JQ393672 ^G	KY492643	KY495268	KY492662
<i>Dendrocitta cinerascens</i>	KUNHM 17738	Malaysia: Sabah	KY495248	KY492622	KY492611	KY492644	KY495269	KY492663
<i>Dendrocitta formosae</i>	KUNHM 23470	Vietnam: Dien Bien; Muong Nhe Nature Reserve	KY495249	KY492623	JQ864494 ^G	KY492645	KY495270	KY492664
<i>Dendrocitta frontalis</i>	KUNHM 15238	Myanmar: Jed Lwe	KY495250	KY492624	JQ864495 ^G	KY492646	KY495271	KY492665
<i>Dendrocitta vagabunda</i>	GenBank		JQ864453	—	JQ864496	KJ454790	—	—
<i>Garrulus glandarius</i>	KUNHM11120	China: Guizhou; Kuan Kuo Shui Nature Reserve	KY495260	KY492625	AB242559 ^G	KY492647	KY495272	KY492666
<i>Gymnorhinus cyanocephalus</i>	GenBank		AY030141	AY030161	AY030115	AY395589	DQ912651	—
<i>Nucifraga caryocatactes</i>	KUNHM 25176	China: Szechwan	—	KY492626	KJ456365 ^G	KY492648	KY495273	KY492667
<i>Perisoreus canadensis</i>	LSUMZ 51588	USA: Idaho	KY495251	KY492627	PCU77331 ^G	KY492649	KY495274	KY492668
<i>Perisoreus infaustus</i>	GenBank		—	—	PIU86042	AY395591	AY395608	—
<i>Perisoreus internigrans</i>	GenBank		—	—	AY395621	—	—	—
<i>Pica pica</i>	KUNHM 28683	Mongolia: Gobi Altai; Han Taishir Mountain	KY495252	KY492628	KJ456399 ^G	KY492650	KY495275	KY492669
<i>Platysomus leucopterus</i>	LSUMZ B58660	Malaysia: Sarawak	KY495253	KY492629	KY492612	—	—	—
<i>Podoces hendersoni</i>	KUNHM 20618	Mongolia: Omnogobi; Halzan Mountain	KY495259	KY492630	—	KY492651	KY495276	KY492670
<i>Psittorhinus morio</i>	KUNHM 21842	Mexico: Campeche; Silvitic	KY495254	KY492631	DQ912594 ^G	KY492652	KY495277	KY492671
<i>Ptilostomus afer</i>	GenBank		—	—	PAU86040	AY395594	AY395613	—
<i>Pyrhhorcorax graculus</i>	AMNH DOT11085		JQ864479 ^G	NC_025927 ^G	JQ864522 ^G	—	—	—
<i>Pyrhhorcorax pyrrhcorax</i>	KUNHM 28865	Mongolia: Bulgan Soum; Baitag Boyd Mountain	KY495255	KY492632	JQ864523 ^G	KY492653	KY495278	KY492672

(Continued)

Appendix 1. (Continued).

Genus/species	Institution/ Sample #	Locality	GenBank Accession					
			ND2	ND3	Cytb	Myo2	Fib7	TGF
<i>Temnurus temnurus</i>	GenBank		–	–	AY395626	AY395596	AY395614	–
<i>Urocissa caerulea</i>	NTNU		KY495256	KY492633	–	KY492654	KY495279	KY492673
<i>Urocissa erythrorhyncha</i>	KUNHM 11247	China: Dashahe Nature Preserve	KY495257	KY492634	KJ456508 ^G	KY492655	AY395615 ^G	KY492674
<i>Urocissa flavirostris</i>	KUNHM 15139	Myanmar: Ma Jed	KY495258	KY492635	JQ864528 ^G	–	–	KY492675
<i>Lanius collaris</i>	GenBank		AY529960	JQ239269	EF635018	HQ996796	–	HQ996870
<i>Lanius collurio</i>	GenBank		–	–	–	–	AY395617	–

Notes: GenBank sequences from previous studies are denoted by ^G. Institutional abbreviations: AMNH, American Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; NTNU, National Taiwan Normal University.

Appendix 2.

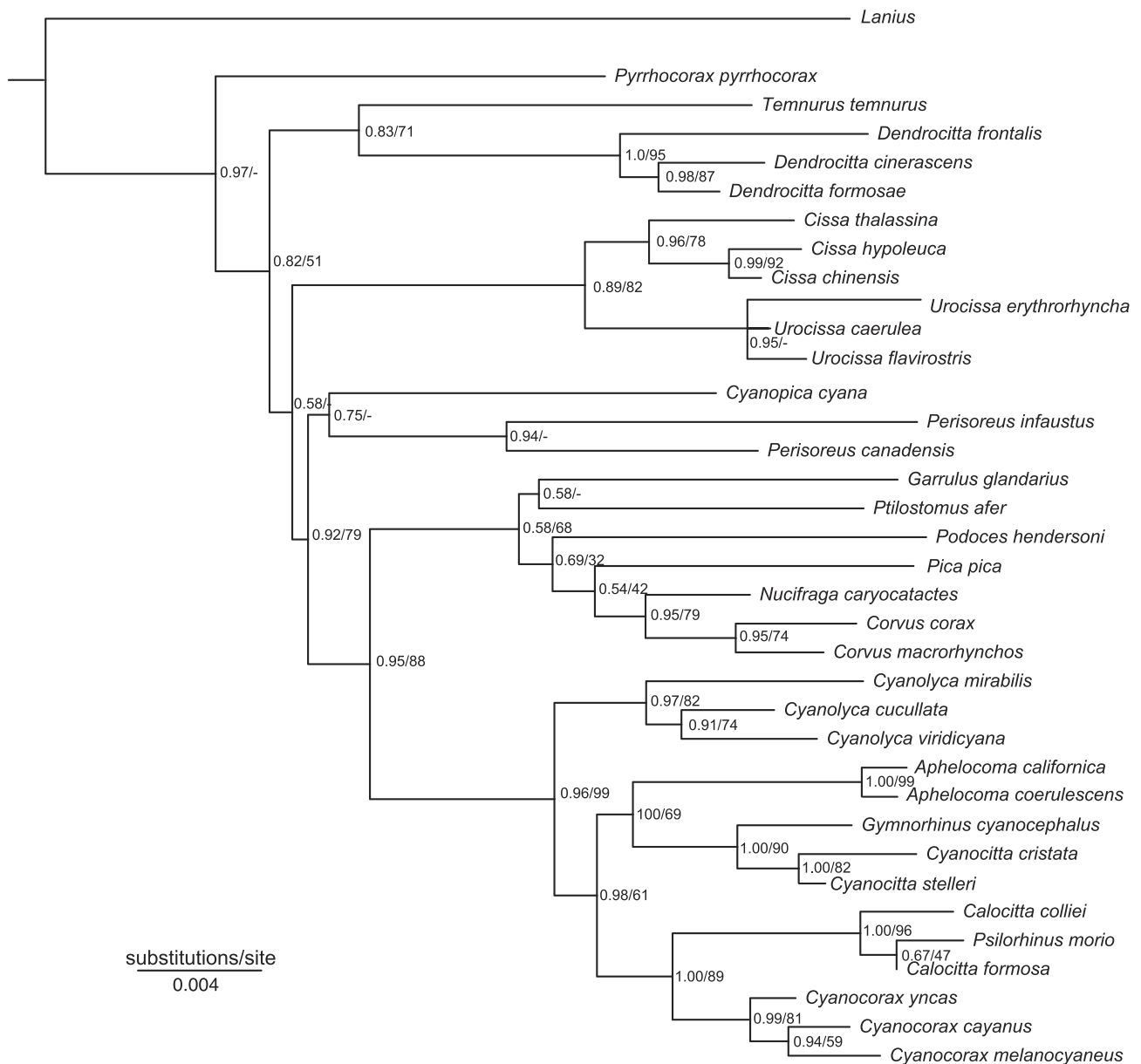


Figure 2A. Phylogeny based on Bayesian and maximum-likelihood analyses of the combined nuclear data set. Numbers at the nodes refer to Bayesian posterior probability/maximum-likelihood bootstrap support.

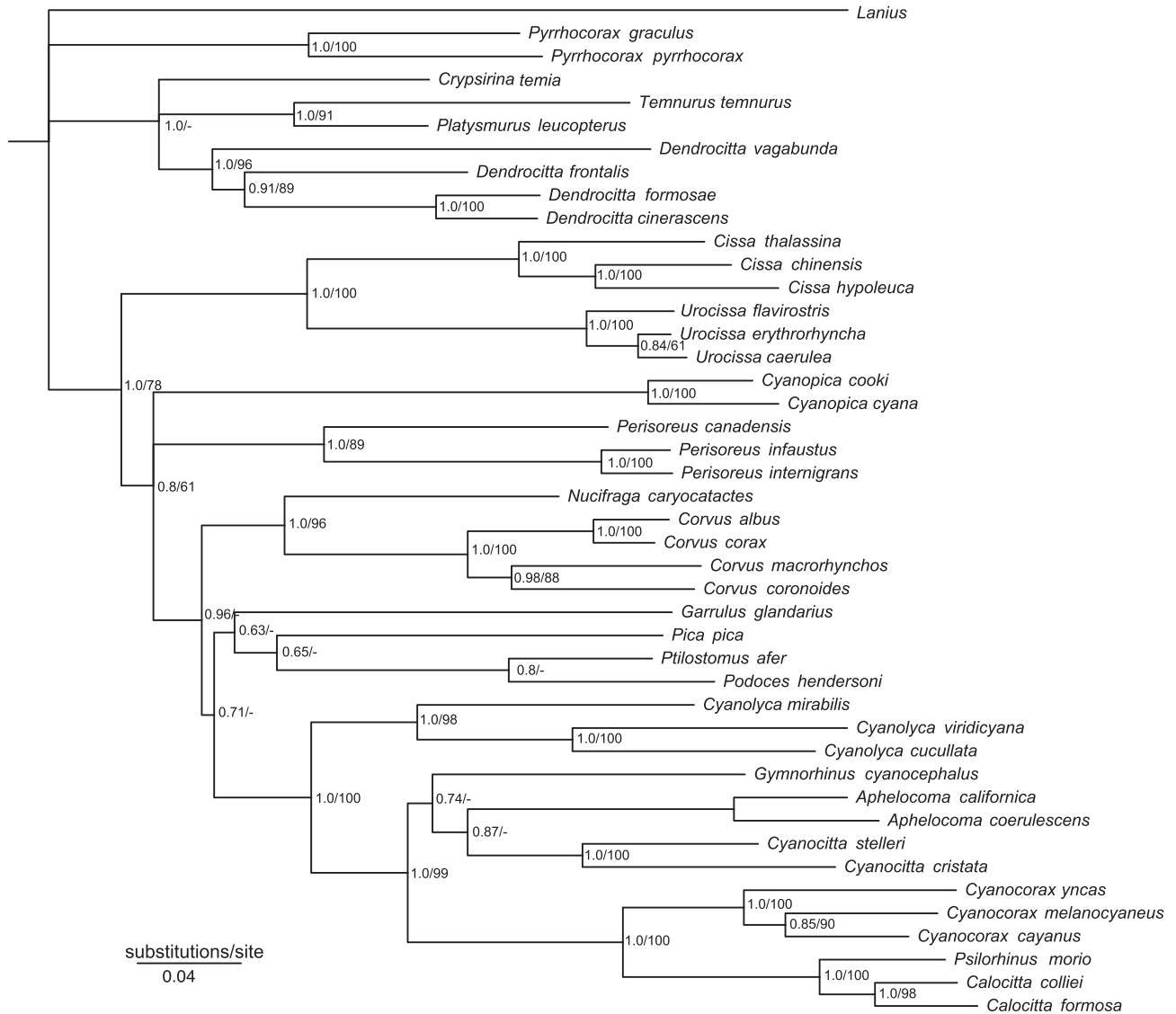


Figure 2B. Phylogeny based on Bayesian and maximum-likelihood analyses of the combined mitochondrial data set. Numbers at the nodes refer to Bayesian posterior probability/maximum-likelihood bootstrap support.